

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representation of
The original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/00		A1	(11) International Publication Number: WO 92/20329 (43) International Publication Date: 26 November 1992 (26.11.92)
(21) International Application Number: PCT/US92/04048 (22) International Filing Date: 13 May 1992 (13.05.92)		(71)(72) Applicant and Inventor: FUISZ, Richard, C. [US/US]; 9320 Cornwell Farm Road, Great Falls, VA 22066 (US). (74) Agent: BARON, Ronald, J.; Hoffmann & Baron, 350 Jericho Turnpike, Jericho, NY 11753 (US).	
(30) Priority data: 702,068 17 May 1991 (17.05.91)		US	(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KR, LU (European patent), MC (European patent), NL (European patent), SE (European patent), US.
(60) Parent Application or Grant (63) Related by Continuation US Filed on 702,068 (CIP) 17 May 1991 (17.05.91)		Published <i>With international search report.</i>	
(71) Applicant (<i>for all designated States except US</i>): FUISZ TECHNOLOGIES LTD. [US/US]; 3810 Concorde Parkway, Suite 100, Chantilly, VA 22021 (US).			

(54) Title: ENZYME SYSTEMS

(57) Abstract

Enzyme products are disclosed. The enzyme products include an enzyme-bearing matrix formed by subjecting a feedstock containing enzyme(s) and carrier materials to conditions which alter the physical and/or chemical structure of the carrier. The matrix suspends the enzyme for protection, delivery, dispersion and activation at the desired time and under selected conditions. Methods of producing the enzyme carrying matrix and enhanced enzyme products are also disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark	MG	Madagascar		
ES	Spain				

1 ENZYME SYSTEMS

BACKGROUND OF THE INVENTION

5 The present application is a Continuation-In-Part of U.S. Patent Application Serial No. 07/702,068 filed on May 17, 1991.

10 The present invention relates to new enzyme products. In particular, the invention relates to improved enzyme products such as leavening agents, alcohol fermenters, detergent ingredients, degradation agents, diagnostic agents, bioremediation agents, 15 catalases and oxidases.

20 Enzymes are proteins which catalyze many biological reactions. As a result of their extraordinary catalytic power and specificity, enzymes have been used to speed up processes that would not otherwise occur. Many 25 isolated enzymes are relatively unstable, often gradually lose activity prior to use, and may be easily inhibited by many factors.

30 Over the years, a number of enzyme products have been developed for a variety of purposes. For example, foods, detergents, cosmetics and pharmaceuticals have all been enhanced by enzymes. Many commercially prepared enzyme-based products, however, have certain 35 drawbacks.

-2-

1 As an illustration, detergent enzymes, are usually
produced in powdered or liquid form. They are difficult
to handle, may cause an irritating dust, may be
5 incompatible with other detergent products, and may
deteriorate in the presence of moisture. The activity
of enzymes in liquid detergents, which contain high
levels of water and surfactants, tends to decrease over
10 time. Frequently, the surfactants inactivate the
enzymes. Consequently, there is a need to prepare
enzyme products suitable for detergents which are easy
15 to handle, do not cause irritation to users, and can be
distributed uniformly in the detergent without reduced
activity.

20 Similarly, it is important to be able to deliver
and activate leavening agents and alcohol fermenters at
the desired time and location in a biomass. For
example, yeast has a tendency to "clump" together in
25 aggregates which resist being dispersed during mixing.
This "clumping" occurs with both dry formulations and
paste formulations of yeast when added to dough or to a
biomass. Thus, it would be beneficial to be able to
30 suspend agents, such as yeast, in a medium for delivery
and release as desired. This is especially true when
the receiving material is an extensive mass, such as
35 dough in baking and the biomass in fermentation
procedures.

-3-

1 Other enzyme-bearing products can benefit from
enhanced shelf-life. At room temperature enzymes used
as indicators in immunoassays frequently experience
5 short shelf-like. Horseradish peroxidase, lipoprotein
lipase, glycerol-3-phosphate oxidase are ordinarily
stored as freeze-dried powders at -20°C. Commonly-used
assays are conducted in the range of 20-30°C. It is
10 thus important to provide a matrix which can improve the
shelf life of enzymes used in immunoassays without
impairing their activity.

15 There have been attempts in the past to deal with
the problems associated with the use of enzymes. In
United States Patent No. 3,095,358, sorbitol is used to
20 stabilize aqueous solutions containing papain, proteases
and amylases. This method requires large amounts of
stabilizing agent and is, therefore, expensive.

25 In U.S. Patent No. 3,296,094, partially hydrolyzed
and solubilized collagen and glycerol are used to
stabilize aqueous solutions of proteolytic enzymes.
This method requires large quantities of glycerol and,
30 therefore, adds significantly to the cost of the enzyme
solution.

35 U.S. Patent No. 3,749,671 discloses a method of
preparing enzyme-containing prills for use in laundry
detergents. The disclosed prilling method requires the

-4-

1 following steps: (a) heating a normally solid
translucent material to a temperature sufficient to melt
the material but insufficient to destroy the activity of
5 the enzyme; (b) forming a slurry of the melted material
and the enzyme; (c) injecting an inert gas into the
slurry to form a uniform dispersion with the gas; and
(d) forming prills from the resulting slurry. This
10 method has many steps which require energy, equipment,
and manual labor.

15 Although the methods discussed above represent
efforts to improve enzyme-containing detergent products,
the problems associated with the decreases in enzyme
activity over time and adequate dispersal have not been
20 solved.

It is, therefore, an object of this invention to
provide an enzyme product which disperses or dissolves
25 uniformly in the target liquid while retaining the
enzyme activity for prolonged periods of time prior to
use.

30 It is another object of this invention to provide a
matrix which facilitates mixing an enzyme with a mass so
that the enzyme can be dispersed efficiently throughout
the mass.

35

-5-

1 It is yet another object of this invention to
provide an enzyme product that exhibits an enhanced
shelf life.

5

Other and further objects of the present invention
will become apparent the following description and its
scope will be pointed out with the appended claims.

10

SUMMARY OF THE INVENTION

15 The present invention includes an enzyme product
which contains a matrix formed by subjecting a feedstock
containing an enzyme and a carrier material to
conditions of temperature and shear sufficient to
20 produce the matrix which suspends the enzyme for storage
and use. The carrier material undergoes transformation
during processing in which its physical and/or chemical
structure is altered.

25

30 "Enzyme product" in the present invention means a
product which includes one or more enzymes. A
nonlimiting list of enzymes which can be suspended in
the matrix includes amylases, proteases, invertases,
glucose oxidases, pectinases, lipases, lactases, and
cellulases. The enzymes make up from about 1% to about
35 30% by weight of the matrix, with amounts of from about
5% to about 25% being preferred and the amounts are from
about 10% to about 20% being most preferred.

-6-

1 Carrier materials which can be used for the matrix
are saccharides, thermoplastic polymers, biodegradable
polymers and water soluble cellulosic materials. The
5 saccharides may be sucrose, lactose, fructose, dextrose,
sorbitol, mannitol, maltose and mixtures thereof. The
saccharides may also be selected from polydextrins,
maltodextrins, and mixtures thereof. Thermoplastic
10 polymers include polypropylene, polystyrene,
polyethylene, polyvinylacetate, polyvinylalcohol, poly
(methyl methacrylate), polyacrylic resins,
lactide/glycolide copolymer and mixtures thereof.
15 Biodegradable polymers include poly(cis-isoprene)
aliphatic polyesters, polyurethanes and urea-
formaldehyde polymers. The cellulosic materials are
water soluble and include methyl cellulose, ethyl
20 cellulose, hydroxymethyl cellulose, ethyl cellulose,
alkali metal salts of carboxy methyl cellulose and
mixtures thereof.

25 As a result of the present invention, enzymes can
be suspended, protected, dispersed and generally
engineered for selective delivery at desired sites under
30 selected conditions. Various enzyme products can be
provided which disperse or dissolve uniformly in the
target liquid, biomass, etc. The enzyme products of
this invention can also be designed to retain their
35 activity for long periods of time prior to use. A non-
inclusive list of uses for the matrix of the invention

-7-

1 includes leavening agents, alcohol fermenters,
detergents, digestive aid products, clinical diagnostic
agents, bioremediation agents, meat tenderizing
5 products, wound debridement and other therapeutical
uses.

10 For a better understanding of the present
invention, reference is made to the following
description and its scope will be pointed out in the
appended claims.

15

DETAILED DESCRIPTION OF THE INVENTION

In the present invention an enzyme-bearing matrix
can be formed by subjecting carrier feedstock and an
enzyme to conditions of temperature and shear to form
20 the matrix. This can be accomplished by melt-spinning
the enzyme with carrier materials. The matrix is
included in various enzyme-based products such as
leavening agents, alcohol fermenters, detergents,
diagnostic agents, degradation products, petroleum
hydrocarbons degraders, digestive aids, therapeutic
25 enzymes, etc.

30

The spinning process can be carried out with
"cotton candy" fabricating-type equipment. The spinning
machine used in the present invention can be a cotton
35 candy-type machine, such as the Econo Floss model 3017
manufactured by Gold Medal Products Company of

-8-

1 Cincinnati, Ohio. It will be appreciated by those
skilled in the art that any apparatus or physical
process which provides similar forces and temperature
5 gradient conditions can also be used. For simplicity in
disclosing and describing this invention, the term
"melt-spinning" will be understood to mean a process
which includes a combination of temperature, shear,
10 flow, flow rate, mechanical forces and thermal gradients
of the type produced by a cotton candy-type machine.

15 The apparatus is operated at a temperature and
speed which induce flash flow of certain carrier
feedstocks without deterioration of the feedstock and
enzyme(s) being processed. The resulting matrix is in
20 the form of a floss, fibre, particle, flake, spicule, or
other generally non-descript aggregate capable of
protectively carrying and delivering an enzyme.

25 The process for producing the matrix includes
introducing a mixture containing an enzyme and a carrier
material simultaneously to conditions of elevated
temperature and shear created by centrifugally forcing
30 the ingredients through orifices. The extremely short
amount of time the ingredients are exposed to the
elevated temperature and shear allows the matrix to be
formed without adverse effects.
35

-9-

1 The flash flow phenomena occurs when a solid
carrier material mixed with an enzyme is subjected to
conditions of melt-spin sufficient to provide internal
5 flow. This condition produces the transformation in
physical and/or chemical structure without degradation
of the material. Internal flow occurs when the
infrastructure of the material breaks down sufficiently
10 to permit movement of material at a subparticle level,
and probably at a molecular level. At a molecular
level, internal flow contemplates the movement of
molecules relative to each other.
15

Internal flow of material is generally associated
with melting point or glass transition point. However,
20 it is contemplated that the combined application of heat
and external force is sufficient to produce flow at
temperatures below the melting or glass transition point
for most compositions.

25 The enzymes dispersed in the matrix are selected
from animal-derived, plant-derived and microbially-
derived preparations. These enzymes can be used as part
30 of a leavening product, an alcohol fermenter, a
detergent, a clinical diagnostic agent or a bioremedient
and possibly mixtures thereof. A nonlimiting list
35 includes amylases, proteases, invertases, oxidases,
catalases, pectinases, lipases, lactases, cellulases and
mixtures thereof.

-10-

1 In one aspect of the present invention, the matrix
may be formed by mixing the carrier material with
degradation enzymes such as cellulases, cutinases,
5 lipases and pectinases and mixtures thereof. Cellulase
sources include those originating in the genera
Trichoderma, Penicillium, Aspergillus, Clostridium, etc.
Additional cellulases can include commercially available
10 products. Such cellulases are capable of degrading the
water insoluble cellulose polymer which is part of the
surface membrane of fruits and vegetables.

15 Cutinase sources include those originating in the
genera Pseudomonas, Fusarium, Botrytis, Ulocladium, etc.
Additional cutinases can include commercially available
20 products. Cutinases are capable of degrading water
insoluble cutin polymer which may be present as part of
the surface membrane of fruits or vegetables.

25 Lipase sources include those originating in the
genera Staphylococcus, Candida, Rhizopus, etc.
Additional lipases can include commercially available
30 products. Such lipases are capable of degrading water
insoluble glycerol components comprising part of the
surface membrane of fruits or vegetables.

35 Pectinase sources include those originating in the
genera Rhizopus, Penicillium, Aspergillus, etc.
Additional pectinases can include commercially available

-11-

1 products. Such pectinases are capable of degrading the
water insoluble pectin components comprising part of the
surface membrane of fruits or vegetables.

5

The enzyme bearing matrix of the invention has many uses. For example, a cellulase matrix may be used to increase the permeability of the surface membrane of 10 fruits and vegetables. The increased water permeability across the surface membrane permits easier delivery of substances such as flavorings, sweeteners, stabilizers and preservatives to the interior of the fruit or 15 vegetable. Additionally, the increased water permeability allows for a more efficient method of dehydration of fruits and vegetables. More importantly, 20 the use of naturally produced degradation enzymes as permeability enhancers replaces the use of chemicals such as methanol, chloroform or alkali metal hydroxides, which, if ingested, pose potential harmful side effects 25 to consumers of fruits and vegetables.

30

35

Another important use for the enzyme carrier matrix of this invention is in the preparation of clinical diagnostics products. A nonlimiting list of active ingredients found in clinical diagnostic products include ascorbic acid oxidase, α -glycerophosphate oxidase, lactate oxidase, uriase, cholesterol esterase, cholesterol ester hydrolase, creatinine amino hydrolase, lipase, glycerol kinase, and mixtures thereof.

-12-

1 The clinical enzyme products contemplated herein
are particularly well-suited for use with the matrix of
the invention when it is desired to disperse the dry
5 powder enzymes in aqueous liquids. It should be readily
apparent to the skilled artisan that all of the active
ingredients may also be provided in dry or lyophilized
form and reconstituted with water prior to use.
10 Compositions of this type are clearly contemplated by
this invention. Clinical diagnostic enzymes carried in
the matrix of the invention can also be incorporated
15 into single-layer or multi-layer analytical elements of
the types known in the prior art.

20 In another aspect of this invention, the matrix may
be used to enhance the shelf-life and activity of
enzymes used in immunoassays. For example, when
horseradish peroxidase was spun with the matrix of the
invention, the enzyme exhibited a longer shelf-life, and
25 became more readily active.

30 Another class of enzyme products according to the
invention are improved detergent enzymes. Detergent
enzymes are known in the art as enzymes which attack
stains or soiled areas of fabrics. Suitable categories
of active detergent enzymes found in improved detergents
35 include proteases, lipases, amylases, and mixtures
thereof. The preferred detergent enzymes are proteases
such as subtilisin and amylases such as those derived

-13-

1 from the bacillus species.

5 The new matrix can be used alone or in combination
with other ingredients as a means for dispersing the
added ingredients throughout the material. For example,
particles, chips, flakes, spicules or combinations
thereof can be used to disperse enzymes which are
10 otherwise relatively non-dispersable because of the
physical characteristics of such materials. Thus, the
matrix of the invention can be used to carry detergent
enzymes to be dispersed more easily and uniformly in
15 other materials present in detergent formulations, such
as surfactants, builders, whitening agents, bleaching
agents and the like.

20 In certain embodiments the enzymes are present in
the host microorganism such as in fungi, bacteria or
algae. Examples of host microorganisms include yeasts,
25 bio-remediation materials and the like.

30 In another aspect of the invention, yeasts may be
melt-spun with selected carrier materials to obtain
enhanced leavening products. Yeasts are single cell
microorganisms containing enzymes which are employed in
large scale fermentation processes. The commercial
35 production of fermented beverages, foods, production of
vitamins, alcoholic fermentation, antibiotic producing
fermentations, all require yeasts or their enzymes to

-14-

1 produce products simpler than the starting material.
Regardless of the substrate used or the chosen
microorganism, industrial fermentations require various
5 nutrients for growth including carbohydrates, nitrogen-
containing compounds, growth factors, vitamins and
minerals. In most fermentations, these nutritional
requirements are met by including, among others, yeast
10 products.

In the production of alcoholic beverages, cereal
grains are the principal raw material. Another
15 important ingredient is malt that is used to produce
amylase. Amylases are organic enzymes that change grain
starch into maltose. In fermentation, zymase which is
produced by yeasts converts the amylase produced maltose
20 into ethyl alcohol and carbon dioxide. *Saccharomyces*
cervisiae is the most common type of yeast used in
alcoholic fermentation to generate zymase.

25 In fermentation processes desired metabolic changes
frequently occur in a narrow temperature and pH range.
Accordingly, to increase product yields, it is important
30 to deliver yeasts having enzymatic activity in a narrow
temperature and pH range. In addition, to optimize
product yields, the yeasts must be rapidly and uniformly
dispersable in the target liquid. Thus, yeasts
35 suspended in the matrix of the invention are easily
dispersable in the nutrient medium and have been found,

-15-

1 in some cases, to be more readily active.

5 In another aspect of this invention, the matrix may
be used to enhance the shelf-life and activity of
enzymes used in yeasts. For example, when Fleischman's
dry yeast was spun with the matrix of the invention, the
yeast exhibited a longer shelf-life and became more
10 readily active.

15 Another significant use for the enzyme carrier
matrix of the invention finds application in the
biodegradation of petroleum hydrocarbons. Many species
of bacteria, fungi and algae have the enzymatic
capability to use petroleum hydrocarbons as food. The
20 bacteria genera most frequently isolated as hydrocarbon
degraders are Pseudomonas, Acinetobacter,
Flavobacterium, Brevibacterium, Corynebacterium,
Arthrobacter. The fungus genera include Candida,
25 Cladosporium, Trichosporium and Rhodotorula. These
bacteria and fungi are present in the environment.
Genetically engineered bacteria which have the enzymatic
capability of degrading several groups of hydrocarbons
30 can also be used as petroleum biodegraders.

35 Using the matrix of the invention, it is possible
to disperse rapidly and uniformly these biodegrading
agents and their nutrients into an otherwise hydrophobic
or immiscible environment. In this manner the microbial

-16-

1 cleanup of oil spills occurs more rapidly.

In yet another aspect of this invention, the enzyme
5 carrying matrix may be used in the field of enhanced oil
recovery. Microbial products, as well as viable
microorganisms, suspended in the matrix may be used as
stimulation agents to enhance oil recovery from
10 petroleum reservoirs. For example, a strain of
Acinetobacter calcoaceticus produces emulsan, a
lipopolysaccharide used to stabilize oil in water
emulsions. *Xanthomonas campestris* is a microbial
15 product producing xanthan, a polysaccharide used as a
water flood thickening agent in oil recovery. Both
these microbial products become easily miscible in
petroleum reservoirs when delivered with the matrix of
20 the invention. The result is enhanced oil recovery.

The ability of microorganisms to use petroleum as
25 food also has detrimental effects. For example,
petroleum fuels cannot become contaminated with water or
microorganisms during storage. Such contamination poses
a serious problem for kerosine based jet aircraft fuels.
30 To diminish this problem, antimicrobials that
concentrate at the oil/water interfaces may be used to
reduce the rate of microbial contamination of
hydrocarbons. Antimicrobial organisms may be delivered
35 at the oil/water interfaces by using the matrix of the
invention.

-17-

1 The carriers used in the invention can be any
material capable of being processed to form a matrix
which can protectively suspend the enzyme for storage
5 and/or selective delivery to the site and/or environment
for release and activation. Carrier materials
contemplated for use may be saccharide based,
thermoplastic polymers, biodegradable polymers, and/or
10 water soluble cellulosic material and mixtures thereof.

A non-limiting list of suitable saccharide carriers
15 include sucrose, lactose, fructose, dextrose, sorbitol,
mannitol, maltose, synthetically-derived saccharide
materials such as polydextrose, and the like and
mixtures thereof. Alternative saccharide materials such
20 as maltodextrins and/or corn syrup solids are also
useful. Please note that for purposes of this
invention, applicant refers to maltodextrins and corn
syrup solids (as defined by the FDA) collectively as
25 maltodextrins.

Suitable thermoplastic polymers can include
30 polypropylene, polystyrene, polyethylene, polyvinyl
acetate, polyvinyl alcohol, poly(methacrylate),
polyacrylic resins, lactide/glycolide copolymer and
mixtures thereof. Suitable water-soluble cellulosic
35 materials can include methylcellulose, ethylcellulose,
hydroxymethyl or ethylcellulose, alkali-metal salts or
carboxymethylcelluloses and the like and mixtures

-18-

1 thereof.

In a preferred embodiment of this invention,
5 maltodextrin has been selected as possessing unique
properties as carrier material for the matrix of the
invention. Maltodextrins are composed of water-soluble
10 glucose-based polymers obtained from the reaction of
starch with enzymes or acid in the presence of water.
The hydrolysis reaction produces a carbohydrate mixture
15 of saccharides having a dextrose equivalence (D.E.) of
less than 40. In one embodiment of the invention, the
D.E. is between 20 and 40. (These maltodextrin products
have been classified by the FDA as corn syrup solids).
In another embodiment, the D.E. is between 10 and 20.
20 The maltodextrins useful in the present invention
include some products sold under the trademark MALTRIN®
by the Grain Processing Corporation of Muscatine, Iowa
or "Dri-Sweet" variety of maltodextrins sold by the
Hubinger Company of Keokuk, Iowa. Such products are
25 available as powders, granules or the like.

The enzyme and the maltodextrin can be combined by
30 physically mixing the two ingredients. Ingredients can
be combined using a blender or any technique known in
the art. The maltodextrin and the enzyme can also be
mixed as a dispersion. The dispersion is formed by
35 contacting the combination of ingredients with an
aqueous medium. Dispersion allows the combination to be

-19-

1 mixed with other materials so that a substantially
homogenous mixture of all ingredients is obtained in the
final enzyme product.

5

EXAMPLES

10 The following examples serve to provide further
appreciation of the invention but are not meant in any
way to restrict the effective scope of the invention.

15

EXAMPLE 1

20 A quantity of Columbo® No Fat Yogurt was placed in
cheese-cloth in a refrigerator for 48 hours permitting
the major portion of the water in the yogurt to drain
out. The drained yogurt was then mixed with 35R corn
syrup solids in the ratio of 1:9. This mixture was
subjected to melt spinning with an Econo Floss® machine
25 yielding a quantity of flakes which were thereafter
maintained unrefrigerated for a period of seven days.

30 At the end of the seven day period, the flakes were
added to skim milk in the ratio of 4 teaspoons of flakes
to 1 cup of skim milk. This mixture was then placed in
a 110°F. environment for 24 hours.

35

A nice yogurt resulted from which it can be
concluded that yogurt can be made in dry form by the
subject process which dry form can be stored and

-20-

1 subsequently reconstituted.

EXAMPLE 2

5

This example was carried out using packets of "Fleischman's" dry yeast available in any grocery store. Two packets of the yeast were mixed with 20 grams polypropylene powder obtained from Aldrich Chemical Co., Inc. After mixing, the mixture was spun in the floss machine producing a fibrous floss.

15

A series of three 1 pint plastic bottles were prepared. Into the first (bottle #1) was placed 10 gm of this floss after first rinsing the floss in tap water. Into the second bottle (bottle #2) was placed an equal weight of the floss but without rinsing. Into the third bottle (bottle #3) was emptied a packet of yeast. To each bottle was added 3 gm sucrose and one-half pint of tap water. Over the neck of each bottle was fastened an elastomeric balloon, and the conditions of the three balloons were observed and noted over a period of 24 hours.

30

It was observed that gas was evolved causing inflation of the balloons to a greater or lesser extent. Measured on a scale of 1 to 5 with 1 being minimal and 5 being maximal, the following relative balloon inflations were noted. For bottle #1 the inflations were about 3

-21-

1 and 4 after, respectively, 2 and 24 hours. For bottle
#2 the corresponding inflations were 2 and 4, while for
bottle #3 the corresponding inflations after 2 and 24
5 hours were 1 and 5.

From the foregoing it was established that the
floss modified yeast was active more rapidly than the
10 original unmodified yeast, that rinsing the floss
accelerated the release of yeast activity from the
floss, and that after 24 hours, whether initially rinsed
15 or not, the floss produced substantially the same amount
of total activity. However, the total gas generated due
to yeast activity derived from the floss was not quite as
great as that provided by the unmodified yeast.

20 The yeast provided in the floss material was easily
handled and ideal for mixing in a substantial mass, such
as a mass of dough in a baking process or a biomass in a
25 formation procedure.

EXAMPLE 3

30 Ten grams of Dri-Vac Lactic culture obtained from
Chris Hansen Laboratories containing Streptococcus
thermophilus and Lactobacillus bulgaricus was mixed with
35 5 grams of corn oil. 85 grams of Maltrin® 365 from
Grain Processing Corporation (GPC) were slowly added to
the mixture while mixing continued until all ingredients

-22-

1 were blended thoroughly. One third of the final mixture
was saved as an unspun control and two thirds of the
final mixture was processed by flash flow in an Econo
5 Floss® spinner at 135-145°C at 3600 rpm to produce spun
flakes.

10 The following culture samples were prepared:

- 10 A. 180 grams of sterilized whole milk with 2.5
 grams of the above spun flakes;
- 15 B. 180 grams of sterilized whole milk with 2.5
 grams of the unspun control mixture; and
- 15 C. 180 grams of sterilized whole milk with 0.25
 grams of the Dri Vac Lactic culture.

20 The samples were cultured in a 40°C water bath
overnight. Sample A resulted in a smooth, firm and
intact mass of yogurt which had a velvety smooth texture
when separated into pieces with a spoon. Samples B and
25 C produced a yogurt which had a coarse, porous texture.
The mass of samples B and C was not as firm as that of
Sample A. The texture of Sample A had much better
mouthfeel than Samples B and C.

30

35 The addition of a proven amount of culture to the
sterilized milk is much easier to obtain with the flakes
than with the original lactic culture. Thus, the
present invention enables the artisan to prepare a
yogurt product more efficiently and with predictable

-23-

1 results.

EXAMPLE 4

5

This example is carried out using packets of Fleischman's active dry-yeast available in grocery stores. The yeast was finely ground in a ceramic mortar and pestle and sieved through 60 and 80 mesh screens. 10 Five grams of the sieved yeast were mixed with 2.5 grams of corn oil. The mixture was then added to 42.5 grams of Maltrin® 365 brand maltodextrin obtained from GPC and 15 mixed until a homogenous yeast mixture was obtained.

20

The yeast mixture was processed by flash flow at 135-140° at 3600 r.p.m. in an Econo Floss® spinning machine producing yeast bearing flakes.

25

Two one-pint plastic bottles were prepared. Into the first (bottle #1) was placed 10 grams of yeast-bearing flakes. One gram of the sieved yeast was placed into the second bottle (bottle #2). To each bottle was added 15 grams sucrose and one-half pint of tap water. 30 Over the neck of each bottle was fastened an elastomeric balloon, the conditions of the three balloons were observed and noted over a period of 24 hours.

35

Observing the inflation of the balloons, it appears that the bottle with the flakes inflated the balloon to

-24-

1 approximately the same extent as the bottle with the sieved yeast. However, the rate of inflation for bottle #1 was less than that for bottle #2.

5

The flakes produced in the present example provided a suitable medium for handling and mixing yeast in large masses such as dough for baking or a biomass undergoing
10 fermentation.

10

EXAMPLE 5

15

95 gr. of Maltrin® 365 obtained from GPC and 0.1 gram of Horseradish Peroxidase obtained from Genzyme Diagnostics were mixed thoroughly by geometric dilution. Five grams of mineral oil was then added slowly while mixing until a uniform mixture was obtained.
20

20

25

The enzyme mixture was processed by flash flow at 135-140°C at 3600 r.p.m. on an Econo Floss spinning unit resulting in light pink flakes.

30

The enzymatic activity of processed and unprocessed enzyme was determined by the method entitled Peroxidase. This method was supplied by Genzyme Diagnostics. The principle of this method is the oxidation of Pyrogallol to Purpurogallin by Peroxidase. Reactivity is determined by time course ultraviolet (UV) spectrophotometry. The reaction rate is determined by
35

-25-

1 the slope between 20 and 30 seconds.

5 The enzyme in the flakes remained active after the
unprocessed enzymes were held at 135°C for one hour and
analyzed for activity. The enzyme in the flake retained
the same level of activity as before incubation while
10 the unprocessed enzymes had lost about 20% of its
activity. Thus, the present invention significantly
enhanced the stability of the enzyme.

15

EXAMPLE 6

20 A 100 gram mixture of Maltrin® 365 from GPC and 10%
w/w of the amylase enzyme Termamyl from Novo Nordisk
was obtained by thoroughly mixing in a mortar and pestle
assembly. The mixture was processed by flash flow at
3600 rpm and 135-140°C using an Econo Floss® spinning
25 unit. The processed material was stored at 5°C until it
was analyzed for enzymatic activity.

30 Thereafter, a sample of the processed flakes and
the unprocessed enzyme were equilibrated in an oven at
100°C to determine stability. After four hours the
enzyme in the flakes had retained substantially all its
original activity while the unprocessed sample had lost
35 over half of its original activity.

-26-

1 The enzymatic activity was determined by the method
entitled "Manual Procedure for Determination of Alpha-
Amylase Activity in Enzyme Preparations and Detergents".
5 This method was provided by Novo Nordisk Bioindustrials,
Inc. The principle of the method is to allow the alpha-
amylase to degrade a starch polymer substrate. Phadebas
10 tablets (Phadebas® Amylase Test, supplied by Pharmacia
Diagnostics) are used. This material is a cross-linked
water insoluble blue colored starch polymer. The tablet
also contains bovine serum albumin and a buffer
15 substance. After the tablet is suspended in water, the
starch is hydrolysed by the alpha-amylase, giving
soluble blue fragments. The absorbance of the resulting
blue solution measured at 620 nm (UV spectrophotometry)
20 is a function of the alpha-amylase activity.

Thus, the present invention produced an enzyme-
bearing flake which remained active under equilibrated
25 conditions set forth above for a longer period of time
than the untreated enzyme.

EXAMPLE 7

30 A 200 gram mixture of the Maltrin® 365 from GPC and
10% w/w of the protease enzyme Alcalase from Novo
Nordisk was obtained by thoroughly mixing in a mortar
35 and pestle assembly. The mixture was processed by flash
flow at 3600 rpm and 135-140°C using an Econo Floss

-27-

1 spinning unit. The processed material was stored at 5°C until it was analyzed for enzymatic activity.

5 Thereafter, a sample of the spun enzyme and the unspun enzyme were equilibrated in an oven at 57°C for 21 hours to determine stability. After 21 hours, both samples retained substantially the same activity as was
10 present in the original spun and unspun sample.

15 The proteolytic activity was determined by the method entitled "Determination of Proteolytic Activity Using Azocasein as a Substrate". This method was provided by Novo Nordisk Bioindustrials, Inc. The principle of the method is to allow the proteolytic enzyme to hydrolyze azocasein for 30 minutes at 40°C. Undigested protein is precipitated with trichloroacetic acid and the quantity of digested product is determined by ultraviolet (UV) spectrophotometry.
20
25

The protease enzyme remained active after flash flow processing for the same period of time as the untreated enzyme.

30 The products and process of the present invention have shown dramatic improvement in enzyme-handling and use art.
35

-28-

1 Moreover, while there have been described what are
presently believed to be the preferred embodiments of
the preferred invention, those skilled in the art will
5 realize that changes in modification may be made thereto
without departing from the spirit of the invention, and
it is intended to claim also changes and modifications
as forward in the true scope of the invention.

10

15

20

25

30

35

-29-

WHAT IS CLAIMED IS:

1. An enzyme product comprising an enzyme-bearing matrix formed by subjecting a feedstock comprising said enzyme and a carrier material to conditions which alter the physical and/or chemical structure of said carrier to form said enzyme-bearing matrix for delivery of said enzyme as desired for said product.
2. The enzyme product of Claim 1, wherein said conditions comprise subjecting said mixture simultaneously to flash heating and applied physical force.
3. The enzyme product of Claim 2, wherein said conditions are created by melt-spinning said feedstock.
4. The enzyme product of Claim 1, wherein said carrier material is selected from the group consisting of saccharides, thermoplastic polymers, biodegradable polymers, and water-soluble cellulosic materials.
5. The enzyme product of Claim 4, wherein said saccharides are selected from the group consisting of polydextrose, maltodextrins, sucrose, lactose, dextrose, mannitol, sorbitol, glucose, maltose and mixtures thereof.

-30-

5

6. The enzyme product of Claim 4, wherein said thermoplastic polymers are selected from the group consisting of polypropylene, polystyrene, polyethylene, polyvinyl acetate, polyvinyl alcohol, poly(methyl methacrylate), polyacrylic resins, lactide/glycolide copolymer and mixtures thereof.

7. The enzyme product of Claim 4, wherein said biodegradable polymers are selected from the group consisting of poly(cis-isoprene), aliphatic polyesters, polyurethanes and urea-formaldehyde polymers.

5

8. The enzyme product of Claim 4, wherein said cellulosic materials are selected from the group consisting of methyl cellulose, ethyl cellulose, hydroxymethyl cellulose, ethyl cellulose, alkali metal salts of carboxy methyl cellulose and mixtures thereof.

5

9. The enzyme product of Claim 1, wherein said enzyme is selected from the group consisting of amylases, proteases, invertases, oxidases, catalases, pectinases, lipases, lactases, cellulases and mixtures thereof.

10. The enzyme product of Claim 9, wherein said enzyme is present in an amount from about 2% to about 40% by weight of the matrix.

-31-

11. The enzyme product of Claim 10, wherein said enzyme is present in an amount from about 10% to about 30%.

12. The enzyme product of Claim 11, wherein said enzyme is present in an amount from about 15% to about 22%.

13. The enzyme product of Claim 1, wherein said enzyme is present in an amount from about 1% to about 10% by weight of said product and said enzyme is a protease.

14. The enzyme product of Claim 1, wherein said enzyme is selected from the group consisting of leavening agents, fermentation agents, biodegradation products, detergent agents, immunoassay agents, clinical diagnostic agents, food digestive aids and therapeutic agents.

5
15. A baking dough comprising the enzyme product of Claim 1, wherein said enzyme is a leavening agent.

16. A fermentation biomass comprising the enzyme product of Claim 1, wherein said enzyme is a fermentation agent.

-32-

17. A yogurt product comprising the enzyme product of Claim 1, wherein said enzyme is contained in a yogurt culture.

18. A detergent formulation comprising the enzyme product of Claim 1.

19. The detergent formulation of Claim 18, wherein said enzyme is subtilisin.

20. A method of preparing an enzyme product comprising:

5 providing an enzyme-bearing matrix formed by subjecting a feedstock comprising said enzyme and a carrier material to conditions which alter the physical and/or chemical structure of said carrier to form said enzyme-bearing matrix for delivery of said enzyme as desired for said product.

21. The method of Claim 20, wherein said conditions comprise subjecting said feedstock simultaneously to flash heating and applied physical force.

22. The method of Claim 21, wherein said conditions are created by melt spinning said feedstock.

-33-

23. The method of Claim 20, wherein said carrier material is selected from the group consisting of saccharides, thermoplastic polymers, biodegradable polymers and water soluble cellulosic materials.

24. The method of Claim 23, wherein said saccharides are selected from the group consisting of polydextrose, maltodextrins, sucrose, lactose, dextrose, mannitol, sorbitol, glucose, maltose, and mixtures thereof.

5

25. The method of Claim 23, wherein said thermoplastic polymers are selected from the group consisting of polypropylene, polystyrene, polyethylene, polyvinyl acetate, polyvinyl alcohol, poly (methyl methacrylate), polyacrylic resins, lactide/glycolide copolymer and mixtures thereof.

5

26. The method of Claim 23, wherein said biodegradable polymers are selected from the group consisting of poly(sis-isoprene), aliphatic polyesters, polyurethenes and urea formaldehyde polymers.

5

27. The method of Claim 23, wherein said cellulosic materials are selected from the group consisting of methyl cellulose, ethyl cellulose, hydroxymethyl cellulose, alkali metal salts of carboxy methyl cellulose and mixtures thereof.

-34-

28. The method of Claim 20, wherein said enzyme is selected from the group consisting of amylases, proteases, invertases, oxidases, catalases, pectinases, lipases, lactases, cellulases and mixtures thereof.

29. The method of Claim 28, wherein said enzyme is present in an amount from about 1% to about 30% by weight of the matrix.

30. The method of Claim 29, wherein said enzyme is present in an amount from about 5% to about 25%.

31. The method of Claim 30, wherein said enzyme is present in an amount from about 10% to about 20%.

32. The method of preparing an enzyme product according to Claim 20, wherein said enzyme is selected from the group consisting of leavening agents, fermentation agents, biodegradation products, detergent enzymes, immunoassay agents, clinical diagnostic agents and food digestive aids.

33. A method of preparing a detergent comprising combining an enzyme-bearing matrix formed by subjecting a feedstock comprising an enzyme and a carrier material to conditions which alter the physical and/or chemical structure of said carrier to form said enzyme-bearing matrix for delivery of said enzyme as desired with detergent ingredients to provide said detergent.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/04048

A. CLASSIFICATION OF SUBJECT MATTER

IPC(S) : A61K 31/00

US CL : 424/78.08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/78.08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A 4,335,232 (Irwin) 15 June 1982, entire document.	1-33
Y	US,A, 4,072,658 (Okamoto et al.) 07 February 1978, entire document.	1-33
Y	US,A, 4,855,326 (Fuisz) 08 August 1989, entire document.	1-33
A	US,A, 4,871,501 (Sugimoto et al) 03 October 1989, entire document.	1-33

 Further documents are listed in the continuation of Box C. See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be part of particular relevance
- "B" earlier document published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

20 JULY 1992

Date of mailing of the international search report

27 AUG 1992

Name and mailing address of the ISA/
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

RAJ BAWA

Facsimile No. NOT APPLICABLE

Telephone No. (703) 308-2351

THIS PAGE BLANK (USPTO)